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ORIGINAL ARTICLE

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Distribution of kerosene components in rats following dermal exposure

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Abstract The systemic distribution of kerosene components in blood and tissues was analysed in rats following dermal exposure. Four types of trimethylbenzenes (TMBs) and aliphatic hydrocarbons (AHCs) with carbon numbers 9–16 (C_9–C_{16}) were analysed as major kerosene components by capillary gas chromatography/mass spectrometry (GC/MS). The kerosene components were detected in blood and all tissues after a small piece of cotton soaked with kerosene was applied to the abdominal skin. The amounts of TMBs detected were higher than those of AHCs. Greater increases in TMB levels were found in adipose tissue in an exposure duration-dependent manner. The amounts of TMBs detected were only at trace levels following post-mortem dermal exposure to kerosene. These findings suggest that kerosene components were absorbed percutaneously and distributed to various organs via the blood circulation. Post-mortem or ante-mortem exposure to kerosene could be distinguished when the exposure duration was relatively long. Adipose tissue would seem to be the most useful for estimating the degree of kerosene exposure.

Keywords Kerosene · Trimethylbenzenes · Dermal exposure · GC/MS · Systemic distribution

Introduction

Kerosene is a middle distillate oil product [1] and generally used around the world as a fuel. Kerosene stoves are widely used as house heating devices in Japan and accidental contact with kerosene often occurs in winter. Kerosene which consists of thousands of different hydrocarbons, chiefly consists of 80% AHCs in the range of C_9–C_{16} and 20% aromatic hydrocarbons [2].

Kerosene generally demonstrates relatively low acute toxicity, but sometimes causes death in conjunction with other factors. Analysis of the kerosene components in body samples from a victims is considered useful to determine whether or not exposure occurred in cases where kerosene was found at the scene of crime or accident. A number of studies have utilised the measurement of components in petroleum products in forensic cases [3, 4, 5, 6] and in animal experiments [7, 8]. These studies, however, have been limited to exposure from inhalation.

Dermal absorption is another major route of exposure to kerosene [9]. There are few studies reporting kerosene levels following dermal exposure [10] and no studies on the distribution of kerosene in tissues, although toxicity through dermal exposure has been evaluated in humans [11, 12, 13, 14] and in animal experiments [15, 16, 17].

The current study evaluates the tissue distribution of kerosene components in rats following dermal exposure. The effects of post-mortem exposure, exposure duration and post-exposure time on kerosene levels in biological samples were experimentally investigated with a practical human case.

Materials and methods

Reagents

Standard kerosene was obtained from Shell Petroleum (Tokyo, Japan). Standard AHC (C_9–C_{16}) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Four types of TMBs (cumene, mesitylene, 1,2,3-trimethylbenzene and pseudocumene) and *o*-xylene-d_{10} used as internal standard (IS) were purchased in analytical grade from Wako Pure Chemical (Osaka, Japan). All other reagents were of analytical grade. Standard kerosene, AHCs, TMBs and IS were diluted with ethanol for GC/MS assay. The IS was made at 25 and 100 μg/ml for blood and tissue samples, respectively. AHCs and TMBs were made at 0.5–100 μg/ml for the calibration curves.

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Sample preparation

Whole blood samples (0.5 g) spiked with 1 µl of IS were extracted with 7 ml of *n*-pentane and concentrated to approximately 80 µl under a stream of nitrogen. A 1 -µl aliquot was injected onto a GC/MS system. Tissue samples (0.5 g) were weighed, cut into small pieces, added to 2 ml of distilled water and spiked with 1 µl of IS. The subsequent extraction procedures were the same as for blood samples.

GC/MS assay

Kerosene components were determined by GC/MS, Hewlett-Packard 5972A system (Santa Clarita, Calif.) utilising our previous method [18] with minor modifications. Scan mode (m/z 20–200 every 2 s) was used for identification of the chemicals and selected ion monitoring (SIM) mode for quantification.

Animal experimental procedures

Male Wistar rats weighing 250–300 g (SLC, Shizuoka, Japan) were used. The experimental protocols were approved by the Shimane Medical University Animal Experimental Committee.

Dermal exposure to kerosene was performed as follows: animals were anaesthetised with pentobarbital (Nembutal®, Abbott Laboratories, North Chicago, Ill.) during the treatment. The abdominal fur was clipped and a piece of cotton (2×2 cm) soaked with 1 ml of standard kerosene was applied for 1, 3 or 6 h. The cotton was covered with impermeable laboratory stretch film and fastened with surgical tape. The rat was placed in an air current during the exposure to prevent possible inhalation of kerosene. The skin was washed after the treatment to prevent prolonged exposure. The animals were sacrificed at 0–12 h post-exposure by an overdose of pentobarbital. Blood and tissues (brain, lung, liver, kidney, spleen, thigh muscle and adipose) were harvested and stored at –80°C until the assays.

The study consisted of two experiments. Experiment 1 was carried out to determine whether AHCs and TMBs were absorbed through the skin by 1 h of dermal exposure and the rat was sacrificed immediately (*n*=3). Three rats were sacrificed without dermal exposure as controls, the tissues were divided into two and one half was extracted directly as a negative control and the other half was spiked with 50 nl of kerosene at extraction as a positive control. To exclude any effects derived from the biological materials, 50 nl of kerosene was extracted without biological materials.

Experiment 2 was carried out to determine the effects of post-mortem exposure, exposure duration and post-exposure time on TMB levels in tissues by randomly dividing 44 rats into 4 groups and treating them as indicated in Table 1.

A practical human case

A 77-year-old female who was living alone was found unconscious in her home and transported to hospital. Her general condition was as follows; Glasgow Coma Scale E3V5M6, heart rate 80 bpm, blood pressure 110/80 mmHg, body temperature 29.9°C. She had erythema with blisters on her back, right chest, abdominal wall, right upper arm and bilateral thighs, indicating second-degree burns over 25% of the body surface. There was a strong smell of some kind of petroleum on her clothes. Subarachnoid haemorrhage was found by computed tomography and rupture of the aneurysm at the anterior communicating artery was revealed by angiography in the first few hours. The primary questions the doctors had were:

1. What is the chemical on her clothes?
2. Did the chemical induce unconsciousness?
3. What is the blood level of the chemical?
4. How severely did the chemical effect her tissues?

To elucidate these questions, blood samples were collected immediately after admission and after 3, 42 and 210 h. These samples were then transferred to our laboratory.

Table 1 Animal experimental group in experiment 2

Group	n	Condition at exposure	Duration of exposure [h]	Post-exposure time of sampling [h]
I	20	Alive	1	0, 1, 3, 6, 12
II	4	Alive	3	0
III	4	Alive	6	0
IV	16	Dead	1	0, 1, 3, 6

Results

The mass chromatograms obtained from kidney samples are shown in Fig. 1. The chromatogram of the spiked sample (Fig. 1 top) was almost identical to that of standard kerosene (data not shown). The proportion of kerosene components was different between the spiked samples (Fig. 1 top) and dermal exposure samples (Fig. 1 bottom).

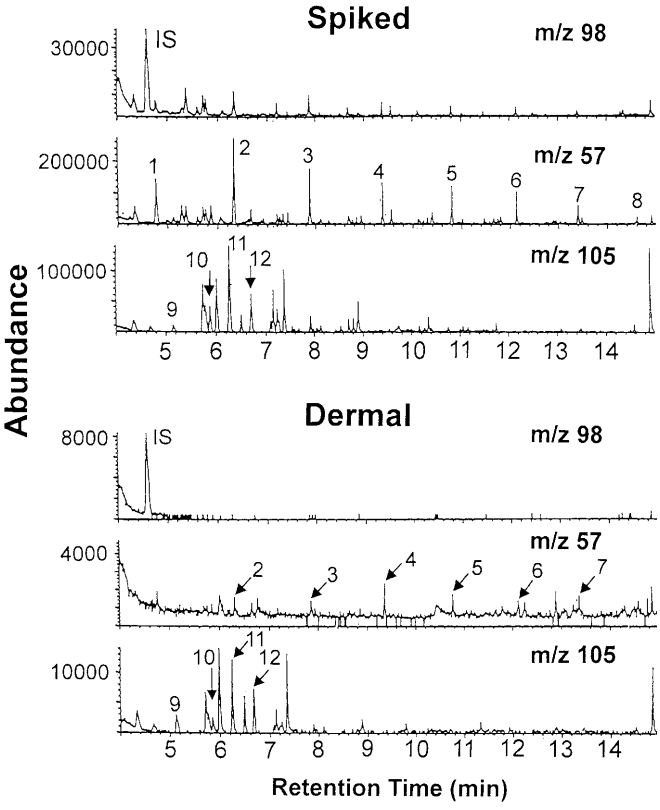

Fig. 1 The mass chromatograms of kidney tissue spiked with 50 nl of kerosene (*top*) and a kidney from a rat which received dermal exposure to kerosene for 1 h (*bottom*). M/z 98, 57 and 105 were monitored for *o*-xylene-d_{10} (IS), AHCs and TMBs, respectively. (*Peak 1)* C_9, *(2)* C_{10}, *(3)* C_{11}, *(4)* C_{12}, *(5)* C_{13}, *(6)* C_{14}, *(7)* C_{15}, *(8)* C_{16}, *(9)* cumene, *(10)* mesitylene, *(11)* pseudocumene, *(12)* 1,2,3-trimethylbenzene

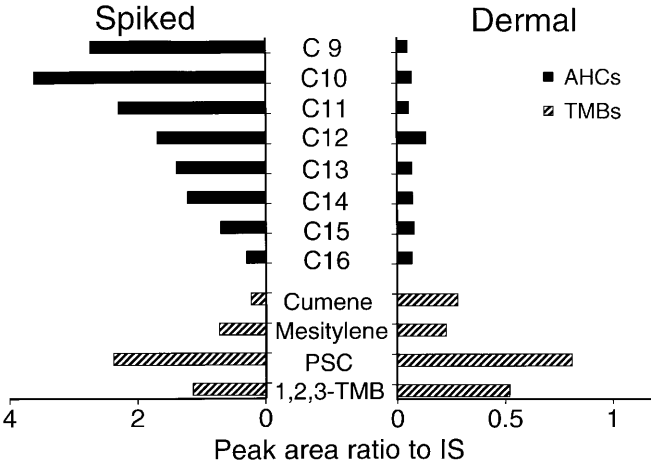

Fig. 2 Peak area ratios of each component to IS in kidney samples are shown. The left bars show the extract from a kidney spiked with 50 nl of kerosene at extraction. The right bars show the kidney extract from the rats which received 1 h of dermal exposure to kerosene. The amounts of trimethylbenzenes (TMBs) were greater than those of aliphatic hydrocarbons (AHCs) in the dermal exposure samples. Data represent mean values. (*PSC* pseudocumene, *1,2,3-TMB* 1,2,3-trimethylbenzene)

The peak area ratios of each component to the IS in kidney samples are shown in Fig. 2. The peak area ratios of AHCs to IS versus the ratios of TMBs to IS in each tissue are shown in Table 2. AHC levels were significantly higher than TMB levels in the spiked samples ($p<0.001$), but significantly lower in dermal exposure samples ($p<0.05$). These results indicate that TMBs were absorbed to a greater degree than AHCs through dermal exposure to kerosene.

TMBs in kerosene were focused upon in experiment 2. The levels of pseudocumene (PSC), one of the major TMBs, at 0 h post-exposure in groups I and IV (see Table 1) are shown in Fig. 3. PSC levels in the rats that received ante-mortem exposure were significantly higher than those in rats which received post-mortem exposure. PSC levels in most of the tissues decreased with the lapse of time post-exposure (groups I and IV), but PSC levels in adipose tissue by ante-mortem exposure (group I) increased up to 3 h post-exposure ($p<0.05$) and then decreased (data not shown). PSC levels were positively correlated to the exposure in a duration-dependent manner in adipose tissue ($p<0.001$ in linear regression), but not in other tissues (0 h in groups I, II and III, data not shown). Similar results were obtained for the other three TMBs (data not shown).

In the human case, the petroleum product on the clothes was determined to be kerosene by detection of the range of AHCs (C_{10}–C_{17}) and major TMBs. The time profiles of major TMB levels are shown in Fig. 4. The TMB levels at 0 and 3 h were similar to the blood levels obtained from 1-h dermal exposure in rats in this study. Further comprehensive discussion of the toxic effects on tissues was difficult since no tissue data was available in this case. A subarachnoid haemorrhage was diagnosed as the primal cause of unconsciousness.

Discussion

The systemic distribution of kerosene components following dermal exposure in rats was experimentally demonstrated. The major kerosene components, AHCs and TMBs, were absorbed through the skin and detected in blood and tissues. The fractions of TMBs absorbed through the skin were greater in quantity than those of AHCs. These results are in accordance with those reported by Kimura et al. in which the fractions of AHCs detected in blood were much less than those of TMBs in rats that had experimentally inhaled kerosene or gasoline [8]. Our results also accord with those reported by McDougal et al. in which the aromatic components penetrated rapidly and all the aliphatic components were identified in the skin in vitro experiment using JP-8, a kerosene-based fuel [19]. Analysing all the patterns of aliphatic hydrocarbons appearing in the chromatogram together would be useful for identifying the type of petroleum product [3, 5, 6, 7, 8].

Kerosene components were only detected at trace levels following post-mortem dermal exposure (Fig. 3). The accumulation of kerosene components in adipose tissue during the post-exposure period was not observed in the rats that received post-mortem exposure. These results suggest that kerosene is absorbed through the skin and

Table 2 The ratio of TMBs to IS versus the ratio of AHCs to IS in tissues obtained from the positive control (*spiked sample*) and the rats that received 1-h dermal exposure (*dermal samples*)

Tissue	Spiked samples			Dermal samples		
	AHCs	TMBs	*p*-value	AHCs	TMBs	*p*-value
Blood	9.9±1.9	3.6±1.6	0.06	0.3 ±0.06	0.4 ±0.4	0.6
Brain	2.8±0.5	1.2±0.5	0.08	0.05±0.006	0.14±0.05	0.05
Lung	3.8±0.7	1.2±0.5	0.05	0.09±0.01	0.09±0.03	0.8
Liver	2.5±0.6	1.1±0.5	0.20	0.08±0.01	0.3 ±0.09	0.01
Spleen	1.6±0.2	0.7±0.3	0.07	0.06±0.009	0.1 ±0.04	0.2
Kidney	1.7±0.3	1.0±0.4	0.33	0.07±0.009	0.5 ±0.1	0.002
Muscle	5.1±1.0	1.2±0.5	0.02	0.12±0.02	0.09±0.02	0.4
Adipose	4.2±0.7	0.9±0.3	0.01	0.24±0.05	0.15±0.07	0.3
Overall	3.9±0.4	1.4±0.3	0.0002	0.12±0.02	0.21±0.05	0.04

Data represents mean ± S.E. The *p*-value was calculated using two-way factorial ANOVA.

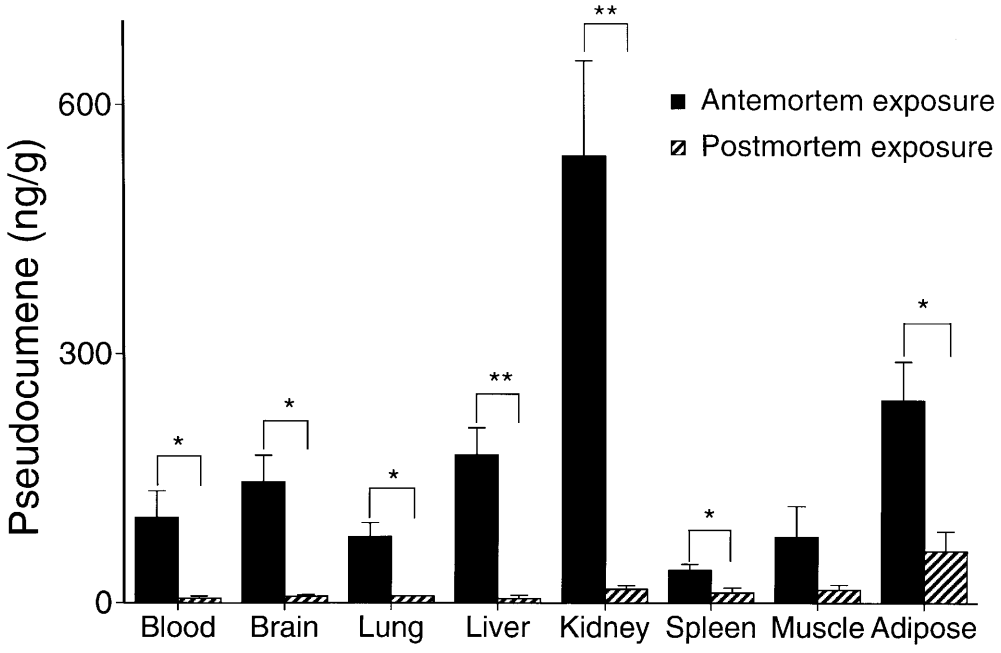

Fig. 3 Pseudocumene levels in rats immediately after 1 h of dermal exposure to kerosene are compared between antemortem (group I) and postmortem (group IV) groups. Data represents mean ± S.E. The data was analysed using two-way factorial ANOVA (*p<0.05, **p<0.01)

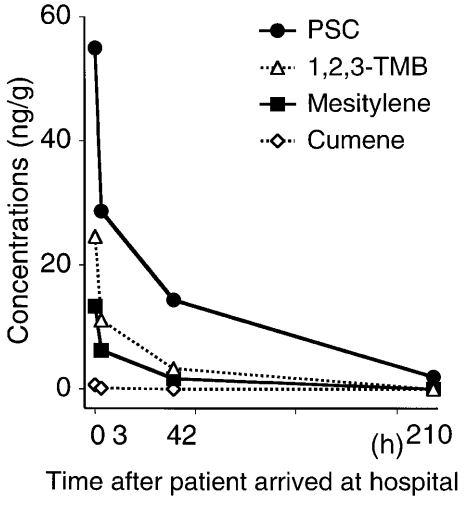

Fig. 4 Time course changes in the concentrations of the four major types of TMBs in the patient's blood

distributed via the blood circulation. Though this study was limited to investigating direct contact to kerosene in open air, the results suggest a usefulness for determination of these components in biological samples to differentiate between ante- and post-mortem exposure [3, 4]. Special attention should, however, be paid to post-mortem redistribution. Takeshita et al. recently demonstrated that postmortem absorption of dichloromethane occurred in both humans and animals in a closed space [20]. Kimura et al. reported significant post-mortem increases in thinner components in tissues [21] and Takahashi et al. demonstrated the effects of subdural haemorrhage on ethanol levels in tissues [22]. The minimal vital period of time to detect kerosene components in tissues from dermal exposure has not been addressed in this study. Further investigations are needed to identify ante- or post-mortem dermal exposure from samples collected after death.

The TMB levels in adipose tissue increased in an exposure duration-dependent manner, but not in other tissues and blood. Accumulation of kerosene occurred in adipose tissue even after exposure was discontinued. It can be assumed that these results occurred because adipose has a high affinity for volatile hydrocarbons and because rearrangement from other body compartments occurs via blood circulation. Adipose would be a useful tissue for forensic investigation when TMB levels are low in other tissues or to estimate the degree of exposure. Further investigation is needed in this area.

In the practical human case, similar TMB levels in blood between the patient and the rats in this study suggest that the protocol used in this study could be a useful model to investigate the kinetics of kerosene components in humans following dermal exposure, nevertheless, a different proportion of exposed skin area on the body surface and a different skin permeability exists between rats and humans [23, 24, 25].

Conclusion

Kerosene components were absorbed and distributed via blood circulation. TMBs were absorbed through the skin to a greater degree than AHCs. Kerosene components in tissues were detected at trace levels following post-mortem exposure. Adipose is a feasible tissue to estimate the degree of exposure.

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